Attempts to develop a simple, objective test for oestrus in sows

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Abstract: To evaluate the accuracy of various techniques for determining the sexual state of sows, four multiparous Landrace sows were housed in stalls 2-5 weeks after weaning. Catheters were inserted under anaesthetic into a prominent ear vein on each sow. Four days later blood and urine sampling commenced, along with other measurements to assess the oestrous state of the animals. Oestrus was detected in all four sows and so were corresponding oestradiol · 17 peaks (22-49 pg/ml) in serum of the three sows from which blood was successfully sampled during the complete 25 · day collection period. Serum progesterone concentrations were highest between days — 5 and — 12 (day 0 = 1st day of standing heat) (peak values of 33 · 1-58 · 2 ng/ml), with values of 3 · 55 ng/ml or less on day 0. Urinary oestrogen was less well correlated with oestrous state than were serum hormone concentrations, but progesterone derivatives in urine corresponded well to serum progesterone with peaks between days —5 and —9. Vulval redness, vulval size, social interest and the occurrence of flehmen were markedly greater during the oestrous period than at other times in the cycle. Body temperature, vaginal pH, the presence of vaginal mucus and behavioural manifestations of oestrus (with the exception of back pressure test) were less well correlated with sexual state. A combination of vulval colour and size, back pressure test, a more detailed study of behaviour and possibly with urinary progesterone derivatives, should give the best indication of the incidence of oestrus in sows

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Attempts to develop a simple, objective test for oestrus in sows

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SUMMARY

To evaluate the accuracy of various techniques for determining the sexual state of sows, four multiparous Landrace sows were housed in stalls 2–5 weeks after weaning. Catheters were inserted under anaesthetic into a prominent ear vein on each sow. Four days later blood and urine sampling commenced, along with other measurements to assess the oestrous state of the animals.

Oestrus was detected in all four sows and so were corresponding oestradiol-17β peaks (22–49 pg/ml) in serum of the three sows from which blood was successfully sampled during the complete 25-day collection period. Serum progesterone concentrations were highest between days −5 and −12 (day 0 = 1st day of standing heat) (peak values of 33·1–58·2 ng/ml), with values of 3·55 ng/ml or less on day 0. Urinary oestrogen was less well correlated with oestrous state than were serum hormone concentrations, but progesterone derivatives in urine corresponded well to serum progesterone with peaks between days −5 and −9. Vulval redness, vulval size, social interest and the occurrence of flehmen were markedly greater during the oestrous period than at other times in the cycle. Body temperature, vaginal pH, the presence of vaginal mucus and behavioural manifestations of oestrus (with the exception of back pressure test) were less well correlated with sexual state. A combination of vulval colour and size, back pressure test, a more detailed study of behaviour and possibly with urinary progesterone derivatives, should give the best indication of the incidence of oestrus in sows.

INTRODUCTION

There have been many reports on steroid hormone concentrations in blood and urine during the oestrous cycle in pigs (e.g. Guthrie, Henricks & Handlin, 1972; Wiel *et al.* 1981; Raeside, 1963; Jones & Erb, 1968) and other species (e.g. Hodges, Czekala & Lasley, 1979; Ensley *et al.* 1982; Short, 1984) and much of this information indicates good correlations between urinary oestrone and oestrus. Likewise, comprehensive studies of the largely well known behavioural and physiological manifestations of oestrus are available (e.g. Perry, 1971; Signoret, 1972; Alexander, Signoret & Hafez, 1974; Anderson, 1974; Hafez, 1974).

However, only about 50% of sows will respond to a back pressure test alone (Signoret *et al.* 1975), especially in the absence of a boar. The current trial was designed to compare hormone concentrations in blood and urine with simple physiological measurements and behavioural observations. Evaluation of the various techniques and determination of their accuracies were attempted, with the objective of finding a simple method for detecting oestrous tendencies in lactating as well as weaned sows.

MATERIALS AND METHODS

Four multiparous Landrace sows (Landschwein) were housed in stalls at the Veterinary Hospital, University of Zurich, 2–5 weeks after weaning. They were fed 3·2 kg/day of a commercial sow diet in two feeds, with free access to water at all times.

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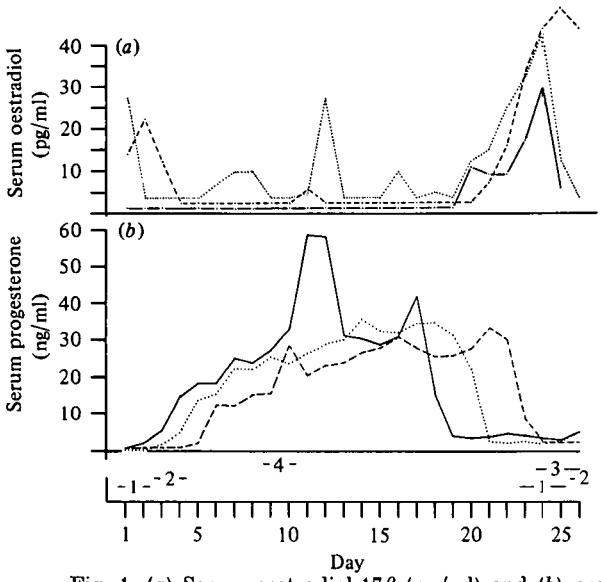

Fig. 1. (*a*) Serum oestradiol-17β (pg/ml) and (*b*) progesterone (ng/ml) in three sows during the oestrous cycle. —, Sow 1; – – –, sow 2; ..., sow 3. Oestrus is represented at the base of the graph, for all four sows.

After missing one meal, and following premedicative sedation with Stresnil (0·5 ml/20 kg live weight), Vetalar (8–10 ml/animal) and Atropin (5–6 ml/animal), anaesthesia was induced with halothane (1–2 %), laughing gas (N_2O) and oxygen (in a ratio of 1 : 1) applied using an open system and mask. A catheter was then inserted into a prominent ear vein, the open end stitched to the ear, the catheter flushed with heparinized saline and then capped. This was then covered with lint for protection. At the start 30 cm long, 1·25 mm diameter Teflon catheters were used. Because of problems with blocking and damage, these were later replaced with 60 cm long, 1·6 mm diameter Portex catheters which proved more satisfactory.

The catheters were flushed again 2 days later with heparinized saline and, 4 days after operating, blood sampling commenced twice daily (08.00 and 16.00 h). Samples were centrifuged and the serum deep frozen prior to radioimmunoassay (RIA) for oestradiol-17β and progesterone. Urine samples were also taken, at least once daily when possible, and were deep frozen prior to RIA for oestrone, oestradiol-17β and progesterone derivatives. As these were spontaneous samples, optical density (using Allen & Rieman's (1953) correction) and urinary creatinine (Jaffa method; Seelig & Wust, 1969) were measured to estimate urine concentration.

The physiological and behavioural assessments also commenced 4 days after the catheters were inserted. These comprised: length, width and depth of the vulva (measured with callipers), vulva colour (scored in comparison with 10 standards; 1–5 light to dark reddish brown, 6–10 light to dark pinkish red), vaginal pH, visible presence or absence and elasticity of vaginal mucus, and rectal body temperature (standardized as a 2 min reading). A back pressure test was attempted daily by applying pressure on both sides of the spine in the lumbar–pelvic region, to see whether sows would adopt the characteristic mating stance. This was then used as the critical criterion for determining whether sows were in oestrus. Sows were let out of their stalls for 5–10 min each day which, as well as encouraging urination, allowed scoring for: their interest in each other and in boars from neighbouring pens, interest in their own and each others' urine and the occurrence of flehmen (as described by Martys, 1977).

RESULTS

Oestrus was detected by positive back pressure test in all four sows. It lasted 2–3 days, with a cycle length of 22–23 days. Blood was successfully collected from three of the four sows over the complete 25-day sampling period, but catheter replacement was necessary for all three. Sow 1 was re-operated on on days 2 and 4, sow 2 on day 14, sow 3 on days 5 and 18 and sow 4 on days 2 (successfully) and 5 (unsuccessfully).

Serum oestradiol-17β and progesterone concentrations are shown in Fig. 1. Oestradiol peaks of between 22 and 49 pg/ml occurred mostly on days -1 to $+1$ (day 0 = 1st day of standing heat), with the exception of sow 3 who showed a peak of 27 pg/ml on day 12. Minima of < 5 pg/ml were found during the rest of the cycle. Progesterone was at a minimum during oestrus (3·55 ng/ml or less) and peak values of 35·4–58·2 ng/ml were between days -12 and -5.

During the 1st week of the trial urine sampling was not complete. No samples were collected on day 1, and missed samples on subsequent days were: sow 1, day 2, 3, 6; sow 2, day 3; sow 3, day 3, 4, 6, 7; sow 4, day 6, 7. Between days 8 and 25 urine was successfully collected daily from all four sows.

Optical density measured urine concentration more reliably than creatinine did, so all urine steroid results are presented as ratios to optical density (Fig. 2). Both oestrone and oestradiol-17β tended to peak around oestrus, but peaks occurred at other times in the cycle too. Urinary progesterone derivatives, with the exception of sow 2 on day 4 (day 1 of her cycle), were consistently low from 3 to 4 days before oestrus until the end of the standing heat.

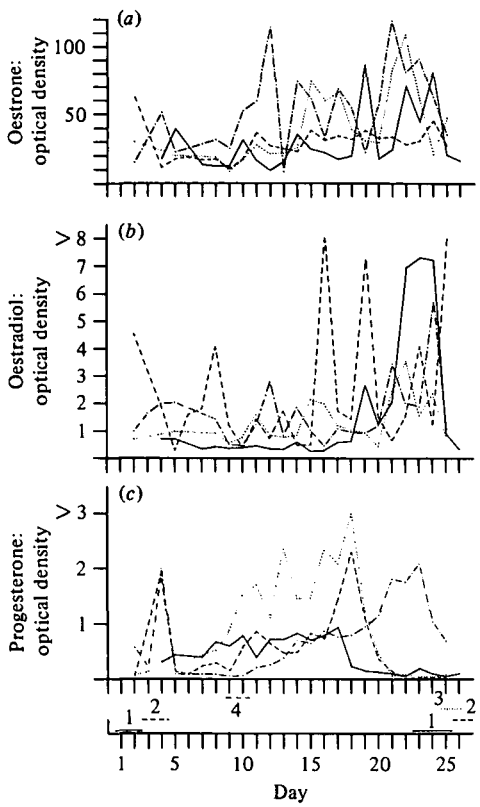

Fig. 2. (*a*) Urinary oestrone, (*b*) oestradiol-17β and (*c*) progesterone derivatives as ratios to optical density for four sows during the oestrous cycle. —, Sow 1; – – –, sow 2; ..., sow 3; –·–, sow 4. Oestrus is represented at the base of the graph.

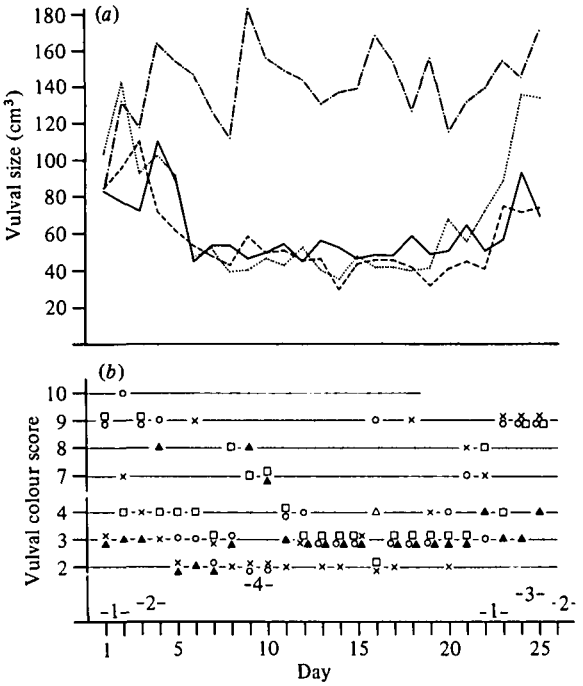

Fig. 3. (*a*) Vulval size and (*b*) vulval colour score of four sows during the oestrous cycle. Oestrus is represented at the base of the graph. —, □, Sow 1; – – –, ○, sow 2; ..., ×, sow 3; –·–, ▲, sow 4.

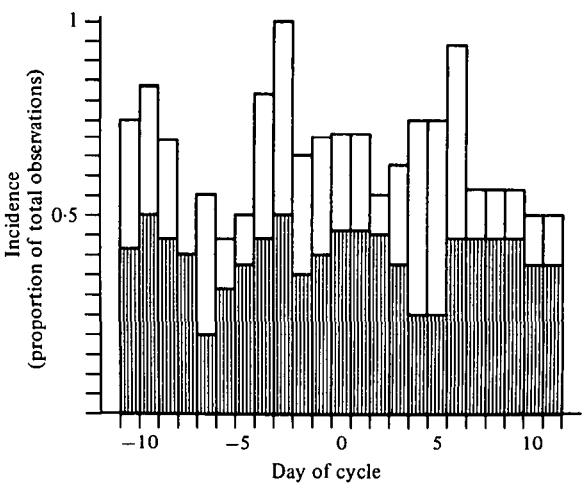

Fig. 4. Observed incidence of flehmen (▢) by four sows and their interest in neighbouring animals (▥), presented as proportions of total observations (day 0 = 1st day of standing heat).

Vulval colour score and vulval size (length × width × depth ÷ 2) (Fig. 3) also showed cyclical changes; the vulva tended to swell and redden during oestrus. Body temperature and vaginal pH were poorly correlated with sexual state and both showed considerable variability within each animal. A score for visibility and/or elasticity of vaginal mucus occurred 12 times between days −1 and +1 and on only two other occasions: days −3 and +3.

Changes in the behavioural indices of oestrus are represented in Fig. 4 by the incidence of flehmen and interest in neighbours (male and female). These are shown as proportions of total observations as the number of observations were not equal for each day of the cycle. There were no marked changes associated with oestrus; nor were there in scores for interest in urine.

DISCUSSION

Oestradiol and progesterone concentrations in serum agreed well with other published data (e.g. Guthrie *et al.* 1972; Wiel *et al.* 1981), although oestradiol was slightly higher and progesterone slightly lower than most other reports. The oestradiol peak shown by sow 3 on day −12 followed

closely after sow 4 was on heat. This could have been in semi-synchronization with her neighbour.

Both urinary oestrogens were considerably more variable than serum hormones and correlated less well with the incidence of oestrus than in other trials (Raeside, 1963; Anderson, 1974; Ensley *et al.* 1982). However, in agreement with Lunaas (1962), minima tended to occur directly after oestrus, particularly for oestrone. As reported by Schomberg *et al.* (1966), a good correlation existed between circulating and urinary progesterone concentrations, with high concentrations from day 7 to 17 of the cycle. The exception to this correlation (a peak in urinary progesterone derivatives in sow 2 on day 4 of the trial; i.e. day 1 of her cycle) could be an error as there was no accompanying serum progesterone peak. It is possible that the sample was contaminated, e.g. with urine from sow 4 which was high in progesterone on that day. It is also likely that there were inaccuracies associated with the collection of spontaneous urine samples (Erb *et al.* 1970), but as the experiment was designed to find a simple method for detecting oestrus in lactating as well as weaned sows, the use of urinary catheters was not feasible.

The lack of evidence for cyclical changes in body temperature and vaginal pH was surprising. Unfortunately there was a wide range in environmental temperature during the sampling period, this and the need to replace catheters in all four sows could have contributed to the high variability of the results. Vaginal mucus, although largely occurring only during oestrus, was not always evident and was thus not very useful as a predictor of sexual state.

Similarly the behavioural scores were highly unreliable as predictors of oestrus, with the obvious exception of the back pressure test. This was probably due to the method of assessment. It is not surprising that sows let out of stalls should show an interest in almost anything, compared with the uninteresting environment in which they are kept. Although the interest scores were poor indicators of oestrus, general observations of the animals could discern differences in sexual state, so there is still some scope for alternative behavioural assessments. The more successful methods of oestrus detection investigated here are now being applied to lactating sows (R. Henderson and A. Stolba, unpublished) to determine the incidence of oestrus and oestrous trends, in combination with a more detailed study of behavioural trends. It is, however, possible that the correlations between these measures are less reliable during lactation.

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